**Olympia oyster larval rearing notes:**

* Collect larvae daily:
  + Catch on 100um screen, pour through 224um to remove debris
  + Rinse with FSW @ 18degC
  + Soak larvae in freshwater @ 18degC for 1 minute
  + Put larvae into tripour, fill to appropriate volume (e.g. 500mL or 800mL), and use pipette to sample ~100 larvae in triplicates to count
  + Count – they should all be live, but note presence of dead.
  + Update collection spreadsheet
* Stock larvae
  + Stocking density calculations:
    - Rule is 1 larva/mL for flow rate = 1 tank turnover per day, so…
    - 4 gallons fill line on buckets = 15 L
    - Flow rate = ~8 L/hr
    - 1 turnover = 1.875 hrs
    - # turnovers per day = 24hrs/1.875 = ~12.8 turnovers per day
    - (12.8 turnovers)\*(1 larv/mL) \* 15,000 mL =192,000 larvae per bucket.
    - **Stock @ 200,000 larvae/bucket**
    - (PSRF: kept under 5mil in 170L conicals)
  + Check the data sheet for allotted daily stocking amount
  + Use plunger to thoroughly mix larvae, then pour into tripour to desired volume.
* Sample extra larvae:
  + Screen larvae onto 100um, carefully pour into Falcon Tube
  + Add 3mL Ethanol, wait for larvae to sink to bottom
  + Remove supernatant to <5mL – can pour off but do not pour out larvae; add 1 mL Ethanol, wait for it to settle
  + Remove supernatant to ~3mL
  + Mix larvae/ethanol with pipette tip, then transfer to vial;
  + Add 1mL ethanol to Falcon tube, rinse larvae off walls with the ethanol, then mix and transfer to vial; wait for it to settle.
  + Carefully remove supernatant from vial.
  + Store in -80
* 1x weekly, clean broodstock
  + Must be done after larvae are collected
  + Stop flow
  + Empty all larval collection buckets
  + Record broodstock location on manifold
  + Record probe location on manifold / in broodstock location
  + Remove broodstock (can keep on wooden dowel), and keep track of table placement - rinse thoroughly with fresh water
  + Empty broodstock buckets & remove outflow tubes, soak in Vortex while cleaning
  + Remove dripper tubes and tube connectors- soak in Vortex while cleaning
  + Rinse, vortex, then rinse all broodstock and larval catchment buckets
  + Replace air stones, wipe outside of air stone tubes with vortex, then rinse
  + Clean shelf
  + Thoroughly rinse and re-install all components: inflow tubes & drippers, bucket outflow tubes, return buckets, larval catchment buckets, and sensors.
  + Return broodstock – place on opposite side and/or end of table at random.
  + Refill buckets, and position air-stones
* 2x weekly, Clean larvae
  + Drop onto 100um screen into water
  + Screen 2 size classes: above 180um, below 180um
    - Can screen on 1 size larger than previous time, to see how larvae are growing!
    - Always have 2 100um “just-in-case I mess up” screens under sorting table
  + Count Live & Dead
  + Calculate Live larvae
  + Clean buckets, replace air stones & drippers
  + Restock in <180um & >180um, combining buckets of **same treatment group** if necessary/space allows
  + Don’t let larvae sit out in tri-pours very long – 15 minutes max.
  + Don’t squish larvae under screen
  + Pour larvae gently, and always into water.
  + While cleaning larvae: rinse lines for 5 minutes with fresh water
* Setting tips: When larvae hold on 224um, try to set them:
  + Provide micro-culch
  + E.g. set on a Tuesday, then on a Thursday before next set, go through and see how everything is doing.
  + No set ratio of micro-cultch/larvae/silo size.
  + Have 2 silos ready per group. Silo A & B. Silo A set on Tuesady, check that next Tuesday and screen through 300um. Silo B set on Thursday, check next Thursday.
  + OR:
    - Set on 450um, wait 2-7 days
      * Could have larvae set on seed if they are in the same tank
      * Should ask Ryan if we could use the coke bottles system…?
  + (Figure out how many larvae I can set in one of those silos. )
  + microculch for singles; 450um can create cluster, BUT this allows
  + *Hazy on the next steps…*
* Making micro-cultch.
  + Find shell-hash pile, screen through to 450, rinse off really well. Stick in micro-wave toaster to dry/sanitize – turn on to max until it looks dry.
* Lessons learned:
  + This year: handled larvae much less and improved production

Boneyard:

* Screen 2-sizes larger.
  + E.g. released 1.5 weeks ago, tank labeled 180
  + Screen on 224 & 200; anything that’s not held on either put back in 180um
  + Always have 2 “just-in-case” screens
  + Screening for size = how quickly are they growing and when to set them? Also, to know when setting tank space is needed.
  + Also, stocking density is dependent on size.
  + Also, size sorting keeps older ones separate from a potentially sick, younger batch.
  + Using screens to size is industry standard. BUT maybe
  + (FYI: Took 9am-2pm to screen out all conicals.)
  + When pouring be careful, gentle, etc.
* Screen size groups for counting purposes - 2 size groups from where they were last measured
* Freshwater bath again? – Nope.
* Clean tanks with vortex
* Rinse lines/algae pump lines with bleachwater, then fresh
  + Run 5 minutes of freshwater, then…
  + 5 minutes with 1mL bleach : freshwater
  + Then run 5 minutes of freshwater again